American Journal of Hematology 52:322–324 (1996)

Different Adhesion Types and Active Sensitivity of Platelet Subpopulations

B. Zhao, T.J. Filler, C.H. Rickert, J.F. Liu, A.D. Skholl-Engberts, and V. Gurevich

Platelet Research and Image Analysis Unit, Institute of Anatomy, University of Münster, Münster, Germany (B.Z., T.J.F., C.H.R.); Department of Surgery, People's Hospital Taiyuan, Taiyuan, Shanxi, People's Republic of China (J.F.L.); Department of Atherosclerosis, Institute of Cardiology, Saint Petersburg, Russia (A.D.S-E., V.G.)

Two different adhesion patterns of blood platelets with different sensitivity to adenosine were demonstrated by means of reflection contrast microscopy with consecutive image analysis and cell affinity chromatography. High-performance liquid chromatography revealed that thrombin-induced serotonin release of adenosine-sensitive platelets was lower than that of adenosine-resistant cells. Our results indicate platelet heterogeneity and suggest that the platelets with lower adenosine sensitivity may be actively involved in the early interaction between platelets and injured endothelium. © 1996 Wiley-Liss, Inc.

Key words: blood platelets, platelet adhesion, heterogeneity, adenosine binding, serotonin

INTRODUCTION

Human blood platelets are intimately involved in hemostasis and atherogenesis. It is generally accepted that platelets adhere to the vessel wall, as they are stimulated by being exposed to injured endothelium [1]. There are studies indicating that only a small part of blood platelets is actively involved in the platelet–vessel wall interaction [2,3]. With respect to heterogeneous morphological and functional characteristics, the existence of different subpopulations of platelets was proposed [4–7]. However, little is known as to whether the adhesion features of platelets and the related biochemical properties are different in such subpopulations. Since platelet adhesion is an early event in platelet activation, it is important to study the hypothesis concerning platelet heterogeneity. It is the aim of the present work to analyse platelet adhesion patterns and investigate the related biochemical nature.

MATERIALS AND METHODS

Preparation of Platelets

Washed human blood platelets were prepared as outlined previously [8]. Briefly, blood was anticoagulated with acid citrate dextrose (8:1 v/v) and centrifuged to obtain platelet-rich plasma. After washing, the platelets were finally suspended in a test medium (pH 7.4) and adjusted to a concentration of 4×10^3 cells/ml.

Reflection Contrast Microscopy

As shown recently [9], 20 μl of platelet suspension was transferred onto Formvar-coated glass. The whole process of settling and spreading of vital platelets was observed by a reflection contrast microscope (RCM). An inverted RCM was used in order to study the contact properties and adhesion patterns of the platelets. The light is reflected by the specimen and causes interference patterns which can be observed and consecutively evaluated by image analysis.

Analysis of Platelet Adhesion

Photoseries were evaluated by the computer-aided gray-scale image analysis system VIDAS 2.5 (Kontron, Munich, Germany). The adhesion patterns of platelets were analysed by measuring their total adhesion area [10].

Received for publication February 20, 1996; accepted March 6, 1996.

Address reprint requests to Dr. B. Zhao, Platelet Research Unit, Institute of Anatomy, University of Münster, Vesaliusweg 2-4, D-48149 Münster, Germany.

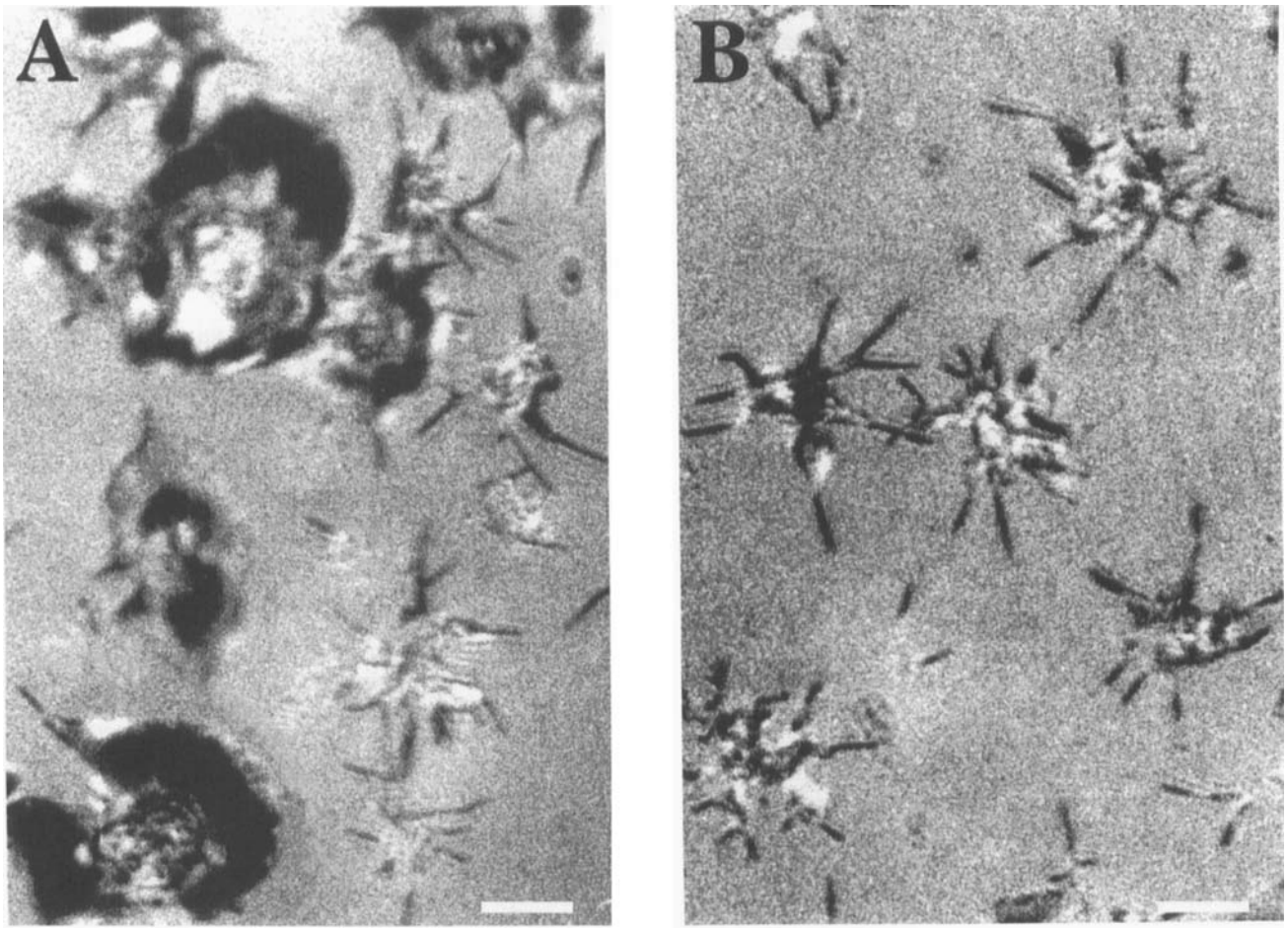

Fig. 1. A: Reflection contrast microscopy of activated platelets settled on Formvar-coated glass. Type A was characterized by flat spreading and extensive homogeneous adhesion zones with occasional short peripheral pseudopodia. Bar = 5 μm. B: Reflection contrast microscopy of activated platelets settled on Formvar-coated glass. Type B showed separate focal and linear contacts in the core area. Pseudopodia occurred regularly and were long. Bar = 5 μm.

Preparation of Platelet Subfractions

Platelet-rich plasma was applied to the column with sepharose CL 2B to collect plasma-free platelets. This cell suspension (25% of column volume) was bound with adenosine–Sepharose [11]. After 5 min of incubation, the elution was started with a flow rate of 1.5 ml/min. The first fraction eluted (32% cells) was considered an adenosine-resistant platelet subpopulation. The elution was stopped, and elution buffer (half-column volume) was layered on top of the affinity support. Sepharose gel was suspended with care inside a column and the elution was resumed. The second eluded fraction was considered adenosine sensitive (45% cells). Nearly 25% of the platelets were almost irreversibly bound with adenosine–Sepharose and could only be removed by replacement with high concentrations of adenosine antagonist aminophyline or dipyridamol.

The release of serotonin, stimulated by thrombin (0.2 U/ml, 30–120 sec), was measured in adenosine-resistant platelets and in adenosine-sensitive platelets by means of high-performance liquid chromatography (HPLC) and expressed as the percentage of the total label in the previously loaded platelets.

RESULTS

Morphology

After sedimentation on Formvar-coated glass, the activation process of vital platelets was observed. In activated platelets two main types of adhesion patterns were found. Type A was characterized by flat spreading and extensive homogeneous adhesion zones with occasional short peripheral pseudopodia. Type B showed separate focal and linear contacts in the core area. Pseudopodia occurred regularly and were long and sometimes dichotomous branched. Both adhesion types coexisted in each sample

but did not change into one another during the observation period of 25 min. The platelets remained stationary and did neither change their location nor their adhesion pattern after the activation process (Fig. 1A,B).

Chromatography

Two platelet fractions, adenosine-resistant and adenosine-sensitive, were eluded from the adenosine–Sepharose column. The number of high affinity adenosine binding sites were $43(\pm 9) \times 10^3$/cell and $101\ (\pm 15) \times 10^3$/cell in adenosine-resistant and adenosine-sensitive subpopulations respectively. K_d values for high-affinity adenosine binding sites in both subpopulations were 24.2 ± 3.1 nM.

Thrombin (0.2 U/ml) induced release of serotonin in both subpopulations. The serotonin release of adenosine-sensitive platelets was lower than that of adenosine-resistant cells ($27 \pm 2\%$ vs. $39 \pm 2\%$, $P < 0.01$).

DISCUSSION

Platelet heterogeneity has been demonstrated with respect to the buoyant density, size, activity of lipid peroxidation, and activity of lactate dehydrogenase [6]. Recently, it has been shown that platelet populations of man and rat could be divided into two classes of about equal size on the basis of presence or absence of an acid phosphatase [4]. An interesting finding of this study, revealed by RCM, are the two distinct adhesion patterns with different adhesive area and different adhesive activity. The different adhesion patterns may be explained as two platelet fractions with different sensitivities to adenosine, since adenosine inhibits platelet activation. It has been shown that intact endothelial cells release adenosine into the bloodstream [12], so that platelets normally do not adhere to intact endothelium [13]. We suppose that the platelets with lower adenosine sensitivity may be actively involved in the early interaction between platelets and injured endothelium. This assumption is supported by the observation that only a small part of the platelets is actively involved in the actions on the vessel wall [2,3].

ACKNOWLEDGMENTS

Helpful discussion with Dr. R. Dierichs, Germany, and technical assistance of Ms. S. Gleis, Ms. M. Holling-Rauss, Ms. P. Hassmann, Ms. A. Lehmann, and Ms. M. Henschkowski are gratefully appreciated.

REFERENCES

1. Aviram M: Platelets and the arterial wall lesion. Curr Opin Lipidol 3:344–348, 1992.
2. Corash L, Tan H, Gralnick HR: Heterogeneity of human whole blood platelet subpopulations. Blood 49:71–87, 1977.
3. Thompson CB, Jacukbovski JA: Pathophysiology and clinical relevance of platelet heterogeneity. Blood 72:1–8, 1988.
4. Behnke O, Forer A: Blood platelet heterogeneity: Evidence for two classes of platelets in man and rat. Br J Haematol 84:686–693, 1993.
5. De Wit LJD, Badenhorst PN, Heyns AD: Ultrastructural morphometric observations on serial sectioned human blood platelet subpopulations. Eur J Cell Biol 43:408–411, 1987.
6. Carty DJ, Gear ARL: Fractionation of platelets according to size, functional and biochemical characteristics. Am J Hematol 21:1–14, 1986.
7. Martin GF, Trowbridge AE (eds): "Platelet Heterogeneity: Biology and Pathology." London: Springer-Verlag, 1990.
8. Zhao B, Dierichs R, Liu B, Berkes P: Gold-labeled low density lipoproteins bind to washed human platelets. Platelets 5:113–120, 1994.
9. Zhao B, Rickert CH, Filler TJ, Liu B, Verhallen PFJ, Dierichs R: Adhesion of washed blood platelets in vitro is advanced, accelerated and enlarged by oxidized low density lipoprotein. Am J Hematol 49:177–182, 1995.
10. Filler TJ, Rickert CH, Zhao B: Evaluation on blood platelets by the image analysis system VIDAS 2.5. Comput Methods Prog Biol 47:177–182, 1995.
11. Gurevich VS, Skholl-Engberts AD: Separation of platelet subpopulations by cell affinity chromatography. Platelets 6:51, 1995.
12. Gerlach E, Becker BT, Nees S: Formation of adenosine by vascular endothelium: a homeostatic and antithrombogenic mechanism. In Gerlach E, Becker B (eds): "Topics and Perspectives in Adenosine Research." Berlin: Springer-Verlag, 1987, p 309–320.
13. Ohisalo JJ: Regulatory functions of adenosine. Med Biol 65:181–191, 1987.